THE FLOWERING OF WOLFFIELLA LINGULATA (HEGELM.) HEGELM.

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The occurrence of flowering in the Lemnaceae has been a subject of great interest during the past ten years. Most of these reduced aquatic plants either flower rarely, or more probably because of their minuteness and obscurity, the flowers are easily overlooked. Flowers in certain species of *Lemna* and *Wolffia,* in some localities at least, are not infrequently observed, while those of *Spirodella* are rarely encountered. Until 1935 flowers of *Wolffiella* were unknown although members of this genus are widespread throughout the warmer parts of the earth and most of the species have been known for approximately seventy years. Because of the failure to find any flowering specimens during this long period, *Wolffiella* has been regarded by some as the most specialized member of the family, having lost completely its capacity to flower. It was supposed that sexual reproduction did not exist and the plants were regarded as being dependent upon vegetative budding as a means of reproduction. Hicks (6) in dealing with this subject states (p. 116–117), "The ability to produce flowers apparently has been so completely lost that probably they are never produced by plants in nature. In *Wolffiella floridana,* at least, it is doubtful as to whether the flowering potentiality could be made to find expression as the result of favorable physiological condition." That *Wolffiella* still retains the capacity to flower is borne out by the finding of flowering material of *W. oblonga* (Phil.) Hegelm. in Argentina and of *W. lingulata* (Hegelm.) Hegelm. in California.

The first species of *Wolffiella* to be reported flowering was *W. oblonga,* a species ranging from South America to North America. Flowers of this species were observed by Giardelli (1) in a lagoon near the town of Dolores, Province of Buenos Aires, Argentina. Careful dissections were made and a very complete diagnosis of the floral characters was drawn up. Illustrations were presented to supplement the descriptions.

In June, 1937, flowering specimens of *W. lingulata* were found in a slough of the marshes of Roberts Island in the delta of the San Joaquin River near Holt, California. On January 11, 1938, a collection of sterile plants was made in Trapper's slough on Roberts Island. These were brought into the laboratory and grown in an aquarium. In three weeks flowers began to appear, and blooming continued over a period of six weeks. In June, 1938, flowering plants were encountered in great abundance at the Roberts Island locality. From these plants seeds developed which germinated freely. On August 26, 1938, many plants were still flowering, although not in such abundance as in June.

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Wolffiella lingulata occurs in quiet water of sloughs in clearings among tules (mostly *Scirpus acutus*). The sloughs are largely the result of excavation for materials to build the dikes which withhold the waters of the San Joaquin river from the rich agricultural delta lands. They have no outlet and obtain their water through seepage from the diked lands as well as from the river. The underlying soil is an immense peat deposit. Associated with the *Wolffiella* were *Riccia fluitans, Typha latifolia, T. angustifolia, Scirpus acutus, Spirodella polyrhiza, Lemna minor, L. cyclostasia, Ceratophyllum demersum, Myriophyllum hippurioides,* and *Jussiaea californica.* Both species of *Lemna* were flowering throughout the summer months.

Wolffiella lingulata was described by Hegelmaier (3) from material collected by Hahn near Mexico City in 1868. A very complete and revised description is given by Thompson (9) which, in certain details, is at variance with the observations recorded here. The vegetative organography has been very ably discussed by Goebel (2). In general this species possesses a very simple rootless thalloid plant body (frond) with a triangular vegetative reproductive pouch at the basal end (pl. XXXVI, fig. 2). The plants are free floating in water at or below the surface but usually do not break through the surface tension layer until the time of flowering. Field observations throughout the entire period of growth and reproduction demonstrate considerable seasonal variation in the size and shape of the fronds as well as in the behavior of the plants. There appear to be two well marked phases of the plant depending upon whether it is in the vegetative or the flowering condition. As in other members of the Lemnaceae the flowering phase in *Wolffiella* is smaller than the vegetative phase (pl. XXXVI, fig. 3). In *Wolffiella,* moreover, it is accompanied by an asymmetrical widening of the basal portion of the frond to accommodate the floral cavity in which the inflorescence is produced. Apparently a reduction in the size of the daughter fronds produced takes place quite generally in the colony just prior to the time of flowering. That there is a connection between flowering and size of the fronds is indicated by the fact that in material growing in the laboratory as well as that in the field, the average size of fronds in colonies producing flowers is much smaller than in those reproducing only vegetatively.

The frond varies from broadly oblong to linear and may range from apparently symmetrical to strongly falciform. There is great variation in proportion of length to width. The length ranges from 1.5 times the width to as much as 7 times the width. Often those that are short have the greatest actual width. The maximum width occurs on vegetative plants. They range from 3.5 millimeters to as much as 5 millimeters wide. Fronds with lengths of 8 or 9 millimeters are not uncommon. Flowering fronds however may be from 3 to 5 millimeters long and as nar-

row as 1 to 1.5 millimeters. Much of this variation in size and shape is seasonal, and descriptions based on a collection taken at any particular season may be very misleading, particularly when applied to a plant from the same locality collected at another season of the year. Except in the flowering condition all of the plants are concave dorsally by virtue of the turning up of the lateral margins. In addition the ends of the fronds are projected downward and only a small portion of the surface area of the plant ever breaks through the surface layer of the water. This curvature of the frond causes it to appear as though it were a "segment of a band." Some of the fronds may also be twisted so as to appear somewhat spiral. The vegetative phase seems to be at its highest development in California during the winter months. At this time the fronds are of maximum size and show the greatest variation.

The most common type of reproduction encountered in *Wolffiella* is vegetative and is accomplished by budding of the meristematic tissue at the inner angle of the reproductive pouch. The young bud is at first strictly symmetrical and is attached to the parent by means of a stipe (pl. XXXV, figs. 7, 8). Goebel regards this as a very degenerate vascular strand. The length to which the stipe will develop shows great variation when correlated with season. Early in the ontogeny of the bud, tissue differentiation takes place and there is formed at one side of its stipe axis a triangular reproductive pouch with its opening facing the parent frond (pl. XXXV, fig. 7). This pouch causes an asymmetrical development of the new frond with respect to its axis as indicated by the position and direction of the stipe. The pouch lies either to the right or the left of the axis. As the frond continues to grow it assumes an apparently symmetrical form, but morphologically the axis is diagonal and follows along one margin of the reproductive pouch. In plants with short stipes the growth of the daughter frond soon causes it to break away from the parent. A definite abscission layer made up of several layers of transverse cells forms across the stipe (pl. XXXV, fig. 8). During the winter months, however, the stipes are very long and often the daughter fronds remain attached to the parent, forming what have been termed family colonies (pl. XXXV, figs. 4, 5). This is contrary to the observations of Thompson who states that in *W. lingulata* family colonies never occur. In material observed during January as many as ten pairs of fronds were seen attached in a family colony each with its stipe sufficiently elongated to accommodate it to colonial existence. Thompson's failure to observe colonies may well be due to the fact that he was dealing with a single seasonal variant with short broad fronds of large proportion. Such size and shape is a definite obstacle to the formation of family colonies. Of this short, broad type of frond not more than five were noted in one family and these families were rare.

The position of the stipe scar on the frond has been used as a diagnostic character for this species of *Wolffiella.* Careful observation of large numbers of plants demonstrates that the stipe scar may be either on the lower margin of the reproductive pouch to the right or to the left of the median axis of the frond or it may be in the exact right or left corner of the frond (pl. XXXV, figs. 9, 10). Both dextral and sinistral fronds are observable in the photograph.

The tissue of the frond is very simple in its structure. It is only two cells thick at the distal end, whereas at the basal end in the region of large intercellular spaces it is several cell layers thick. Such intercellular spaces are characteristic of members of the Lemnaceae. In *Wolffiella lingulata* they occupy only the basal third or half of the frond (pl. XXXVI, fig. 2). The epidermal layers are beset with scattered dense cells which have been termed pigment cells. Upon drying these become red brown. The few stomata (pl. XXXV, fig. 6) that have been observed were on flowering specimens and only on that portion of the frond that is emersed. Goebel reports his inability to see stomata. Hegelmaier noted one or two near the margin of a frond. Although search was made no stomata were seen on strictly vegetative plants. This coincides with the findings reported by Giardelli for *W. oblonga.* Whether or not the development of stomata precedes the emergence of the frond from water has not been observed. In any event just prior to flowering the fronds break through the tension layer of the water surface and soon the development of the inflorescence becomes evident.

The inflorescence occurs in a floral cavity and makes its appearance first as a double colorless spot composed of a dense mass of very small cells on the side of the reproductive pouch that bears the stipe of the frond (pl. XXXVI, fig. 3). Soon it loses its double aspect and appears as a single oblong mass in a floral cavity lying in a plane essentially parallel to the side of the vegetative pouch. Next a slit-like opening occurs in the surface of the frond above the cavity; this is followed by the emergence of the single flask-shaped pistillate flower. Flowering is thus protoginous. When the stigma becomes receptive a small globule of liquid is exuded (pl. XXXVI, fig. 4) which assumes a spheroid form and completely covers the concave stigmatic surface; presumably this is a trap to catch insect or wind borne pollen. With the disappearance of the globule of liquid from the stigma, the staminate flower, composed of a single stamen, begins to emerge. The stamen, lying back of the pistillate flower away from the base of the frond, is made up of a subspheroid two-lobed anther on a stout filament. During its development the stamen usually tears the aperture of the cavity. Soon after emergence it dehisces its dry white powdery pollen and appears as a conspicuous glistening white spot on the surface of the frond

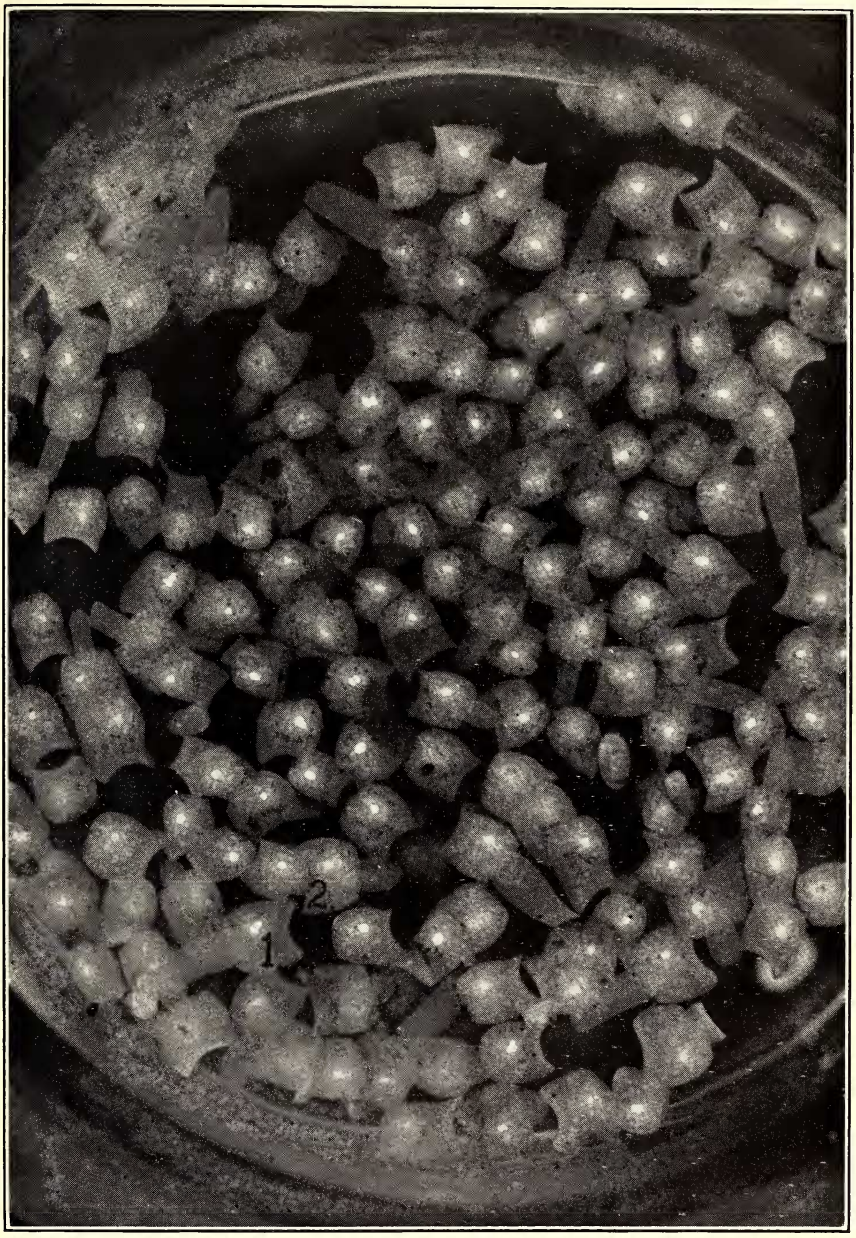

PLATE XXXIV. WOLFFIELLA LINGULATA (Hegelm.) Hegelm. Group of flowering plants, × 5. The shaded portions of the fronds are immersed, the lighter portions bearing the flowers are emersed. (1) Sinistral frond bearing a dehiscing stamen; (2) dextral frond with the pistillate flower in anthesis (dextral and sinistral fronds being interpreted on the basis of the position of the stipe scar rather than the position of the flower).

(pl. XXXIV). It then withers and disappears. There is no evidence of a spathe surrounding the inflorescence.

The young fruit develops asymmetrically into an indehiscent utricle with a single ellipsoid seed and a persistent style (pl. XXXVI, fig. 9). In all plants observed the ripening of the fruit is accompanied by the death of the parent frond, which remains floating with the fruit in place in the ruptured floral cavity (pl. XXXVI, fig. 12). Soon the seed begins to germinate. The ovary wall collapses and disintegrates. The minute brown conical operculum of the testa begins to push out, attached to the hypocotyl (pl. XXXVI, figs. 13–18). As the hypocotyl further enlarges, the operculum becomes increasingly lateral in its position and an irregular slit appears separating the hypocotyl from the cotyledonary sheath. From this slit, the young vegetative frond makes its appearance, the distal end of the frond emerging first. The cotyledon remains within the testa. When fully formed the young plant breaks away from the hypocotyl and other remaining embryo tissues and is a free floating plant with the next vegetative generation already formed in its pouch (pl. XXXVI, fig. 18). At the lower margin of the vegetative pouch the minute stipe by which the young plant was attached to the hypocotyl is clearly visible. A formal description of the inflorescence and flowers of this species follows.

Wolffiella lingulata (Hegelm.) Hegelm. in Engler, Bot. Jahrb. 21: 303. 1895. *Wolffia lingulata* Hegelm. Monogr. Lemnac. 132. 1868. *Wolffiella oblonga* of California authors, *non Wolffiella oblonga* (Phil.) Hegelm. 1857.

Fronds monoecious, protoginous; inflorescence without spathe, borne in floral cavity in dorsal side at basal end of frond, dextrally or sinistrally to vegetative pouch; staminate flower posterior to pistillate flower, stamen one, filament stout, .74–.76 mm. long, anther subspheroid, two lobed, white; pollen white, subspheroid 20–23 microns in diameter, minutely mucronulate; pistillate flower, solitary, pistil flask shaped, .47–.48 mm. high, ovary .13–.20 mm. wide, one-celled, style short, thick, abruptly expanding to a concave circular stigma; ovule solitary, suberect, becoming tilted; fruit a utricle, bladdery, asymmetrical, indehiscent, style persistent; seed ellipsoid ovoid, .41–.44 mm. long, .29 mm. wide, glistening white, operculum of testa lying in a cavity at end of seed (pl. XXXVI, fig. 11).

Specimens from the Missouri Botanical Garden (M) and from the Herbarium of the University of California (UC) were examined.

Mexico. Near Mexico City, *L. Hahn* (type collection, M). California. Kern County: near Bakersfield, Oct. 7, 1895, *C. H. Thompson* (M). San Joaquin County: one-half mile southwest of Holt, *H. L. Mason 11,548* (UC); Trappers Slough, Roberts Island, *H. L. Mason 11,850, 12,072* (UC). San Bernardino

County: near San Bernardino, *Parish 4581* (UC); San Bernardino Valley, *Parish 4586* (UC). Orange County: San Juan Capistrano, *Abrams 4200* (UC). San Luis Obispo County: Oceano, Nov. 14, 1908, *I. J. Condit* (UC). Monterey County: 2 miles south of Pajaro, Sept. 27, 1903, *C. H. Thompson* (M). Santa Clara County: Alviso, Sept. 9, 1903, *C. H. Thompson* (M).

Comparing the above description with that given by Giardelli for *Wolffiella oblonga* the two species are strikingly alike in flower characters. Only two points of difference are outstanding; a third may or may not be significant. For *W. oblonga* there is reported pollen 11 to 15 microns in diameter whereas in *W. lingulata* the pollen is 20 to 23 microns in diameter. In *W. oblonga* the pollen is covered with minute wrinkles whereas in *W. lingulata* it is turgid and minutely mucronulate. In general the size of all the flower parts in *W. lingulata* exceeds that in *W. oblonga.* This is consistent with the larger plant body. The following is a detailed comparison of *W. oblonga* with *W. lingulata* as to points in which measurements were recorded by Giardelli. Except as noted above and in the following table, the flowers agree in other characters. The size of the frond was computed from the magnification cited on Giardelli's illustration.

	W. oblonga	*W. lingulata*
Pollen	11–15 mu	20–23 mu
Pistil	.4 mm. high	.47–.48 mm. high
	.2 mm. wide	.13–.20 mm. wide
Fruit	.45– .55 mm. long	.58–.77 mm. long
	.37– .46 mm. wide	.45–.50 mm. wide
Seed	.35– .40 mm. high	.41–.44 mm. high
	.25– .29 mm. wide	.29 mm. wide
Frond	2.8 –3.5 mm. long	5–8 mm. long

Wolffiella oblonga was first described in 1857 as a species of *Lemna* by Philippi (7) from material collected near Santiago, Chile. Hegelmaier (3) in 1868 in his classical monograph of the Lemnaceae transferred the species to the genus *Wolffia* and erected the subgenus *Wolffiella* to include it and several related species. He expressed the belief that this subgenus should really be considered a distinct genus but was not prepared at that time to make such a disposition of it. However, Hegelmaier (5) later (1895) raised the subgenus *Wolffiella* to generic status and made the combination *Wolffiella oblonga* (Phil.) Hegelm.

The following material of *Wolffiella oblonga* (Phil.) Hegelm. was studied. Chile: Santiago, May, 1857, *Philippi* (type collection, M). Argentina: Cordoba, May 9, 1898, *Stuckert* (M). Uruguay: Montevideo, Pocitas, *Herter 150: 70512* (M, UC).

Wolffiella oblonga (Phil.) Hegelm. has been reported from California, an occurrence based upon two collections from near San Bernardino, by Parish. A careful study of this material and comparison with the type of *W. oblonga* eliminates that species

from consideration in the California flora. These plants are clearly small individuals of *W. lingulata.* It is a matter of interest to note that a single specimen of the Parish collection shows an immature flower in a very early stage of development.

Wolffiella lingulata was described as a *Wolffia,* subgenus *Wolffiella,* by Hegelmaier in 1868. To this in 1878 he added the variety *W. lingulata* var. *minor.* In 1898 Thompson (10) reduced this variety to synonomy with *W. oblonga* (Phil.) Hegelm. When the subgenus *Wolffiella* was raised to generic rank by Hegelmaier in 1896 the combination *Wolffiella lingulata* (Hegelm.) Hegelm. was made.

Wolffiella lingulata, as differentiated from *W. oblonga* by Hegelmaier, was based largely upon size characters. His measure ments for fronds of *W. lingulata* ranged from 4.7 to 6.1 millimeters in length and from 1.8 to 2.4 millimeters in width. For *W. oblonga* he reported for the frond a length of 1.7 to 3 millimeters and a width of .6 to .85 millimeters. Later in describing *W. lingulata* var. *minor* he noted that the size character between the two species broke down, causing him to express doubt as to the specific status of *W. lingulata* as distinct from *W. oblonga.* Hegelmaier noted and figured the difference in the extent of the air cavities in the fronds of the two species but made no particular point of this difference in his diagnosis. This character was given emphasis, however, by J. D. Smith (8) and its importance has been corroborated in the present investigation.

Thompson (9) largely on the basis of field and culture studies of material collected near Bakersfield, California, and supplemented with specimen studies of material from Mexico and South America, laid aside the doubts of Hegelmaier and rediagnosed *W. lingulata,* calling attention to what appeared to him to be the constant position of the stipe scar on the lower lip of the pouch to the right of the median line of the frond. He stated that in *W. oblonga* this scar occurs at the angle of the pouch to the right of the frond. This, he maintained, consistently characterized the small forms of *W. lingulata* as well as the large, while *W. lingulata* var. *minor* on the other hand is in this respect identical with *W. oblonga* and must be regarded as an elongated form of that species. This character, he points out, serves as a ready means of distinguishing these two species. The above observations of Thompson's relative to the position of the stipe scar have not been substantiated in the investigations of the writer. As noted above, both dextral and sinistral fronds have been observed and the position of the stipe scar on the pouch is not a constant character. The dextral and sinistral position of the stipe in all probability is the result of genetic variation within the species and probably only occurs as a result of sexual reproduction. In vegetative reproduction, as has been pointed out by Hegelmaier, the stipe and costa of the daughter frond always develop on the side

of the parent pouch opposite to the position of these organs on the parent (pl. XXXV, figs. 7, 11). Since the base of the daughter frond faces the opposite direction from the base of the parent frond, this necessitates that a dextral frond must produce dextral offspring and likewise sinistral fronds must produce sinistral offspring. This could account for the uniformity of the plants reported by Thompson from a sterile colony. They probably owed their origin asexually to a single migrant individual. The position of the stipe scar on the lower lip of the pouch or on its right hand corner has been found to vary within a family colony, every member of which descended asexually from a single individual. Figures 9 and 10 of plate XXXV are mother and daughter fronds. In figure 10 the stipe scar is on the lower margin of the frond, whereas figure 9 illustrates an individual with the stipe scar at the corner of the pouch. Figure 9 has the characters attributed to *W. oblonga* in this respect. Since it can be demonstrated that the size and shape of the frond is so dependent on local and seasonal conditions the broad short character of the frond attributed to *W. lingulata* by Thompson must be discarded as a basis for differentiating the two species. Likewise the "saber shaped" character attributed to *W. oblonga* by modern writers does not hold. The great majority of the collections of *W. lingulata* through most of the year are saber shaped. Figure 1 of plate XXXV is typical of *W. lingulata* according to the interpretation of Thompson. It is a flowering specimen. Figures 2, 4, and 5 are of the saber shaped type. Flowers were found on both types and no differences other than shape of the fronds were noted between them. The differences between the two species as observed by the writer (pl. XXXVI, figs. 1, 2) will perhaps be best illustrated by a key.

Plants 1.5 to 4 mm. long; angle of vegetative pouch 40 to 50 degrees, the tip somewhat attenuate, the lips strongly rounded; air chambers almost throughout the frond; pollen 11 to 15 microns in diameter, surface minutely wrinkled (*fide Giardelli*) ..... *W. oblonga*

Plants 4 to 9 mm. long; angle of vegetative pouch 60 to 90 degrees, the tip not attenuate; air chambers occupying not more than half the frond; pollen 20 to 23 microns in diameter, the surface muriculate ..... *W. lingulata*

Since two species have been found flowering it would seem that some information should now be forthcoming relative to the generic status of *Wolffiella*. Most writers have made their particular disposition of the group "pending the finding of flowers." In a plant so reduced in the structure of its vegetative and reproductive parts it is scarcely to be expected that conclusive data will be available to serve as a basis for descriptive differentiation. Hegelmaier differentiated *Wolffiella* from *Wolffia* primarily on the position of the axis of the vegetative shoot with respect to the vegetative pouch. In *Wolffia* this shoot lies at the base of the

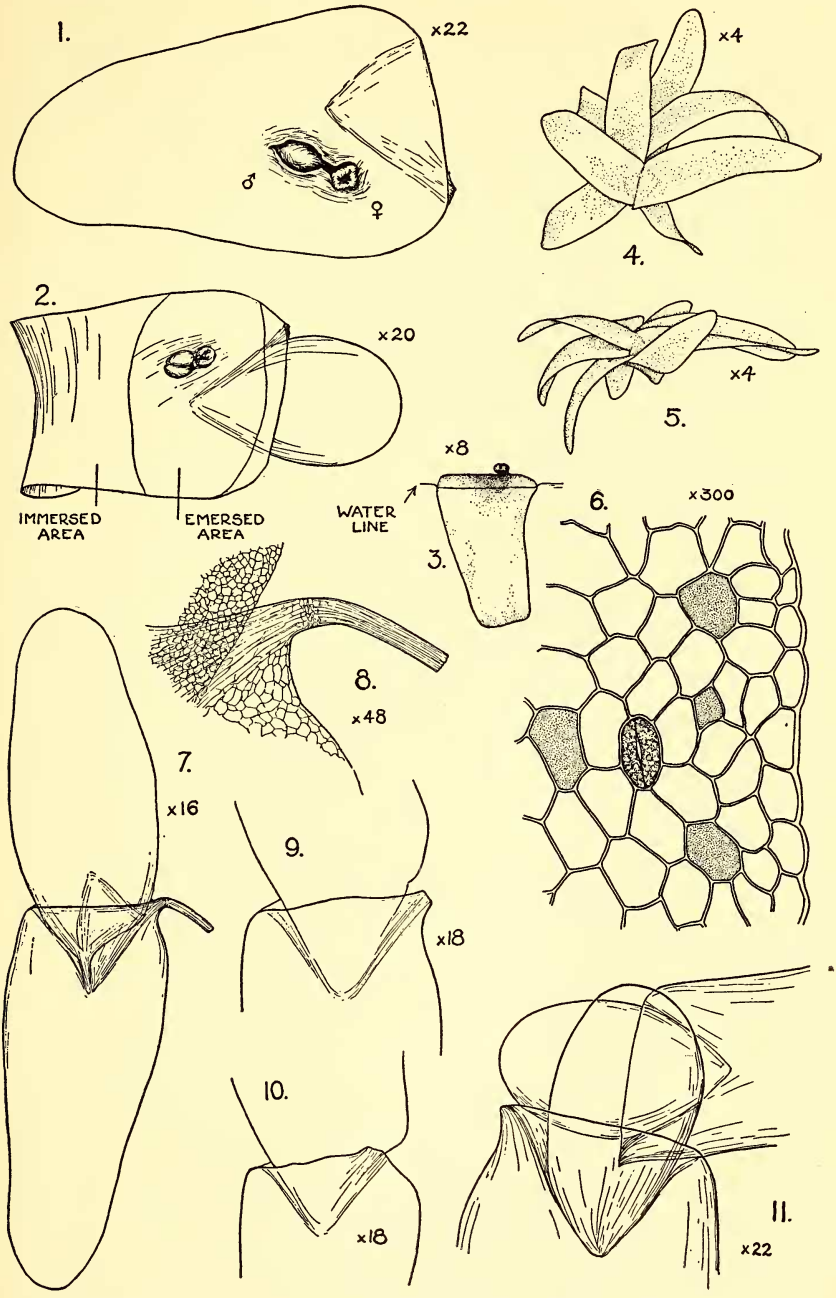

PLATE XXXV. WOLFFIELLA LINGULATA (Hegelm.) Hegelm.
(See explanation of figures on page 250.)